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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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FOREWORD: This report presents the results of the bioassay of L-tryptophan conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: The bioassay of L-tryptophan was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were determined by Drs. D. P. Griswold[1], J. D. Prejean[1], E. K. Weisburger[2], and J. H. Weisburger[2,3]. Ms. J. Belzer[1] and Mr. I. Brown[1] were responsible for the care and feeding of the laboratory animals. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. S. D. Kosanke[1] and J. C. Peckham[1], and the diagnoses included in this report represent their interpretation.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[4]. The statistical analyses were performed by Dr. J. R. Joiner[5], using methods selected for the bioassay program by Dr. J. J. Gart[6]. Chemicals used in this bioassay were analyzed under the direction of Dr. E. Murrill[7], and the analytical results were reviewed by Dr. C. W. Jameson[5]. The structural formula was supplied by NCI[2].

This report was prepared at Tracor Jitco[5] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A. Campbell, Deputy Director for Science; Drs. J. F. Robens and C. H. Williams, toxicologists; Dr. G. L. Miller, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[6]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

The following other scientists at NCI[2] were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Dawn G. Goodman, Dr. Richard A. Griesemer, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire[8], and Dr. Jerrold M. Ward.

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SUMMARY

A bioassay of the amino acid L-tryptophan for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 35 rats and 35 mice of each sex were administered L-tryptophan at one of two doses, either 25,000 or 50,000 ppm, 5 days per week for 78 weeks, and then observed for 26 or 27 weeks. Matched controls consisted of groups of 15 rats or 15 mice of each sex. All surviving rats and mice were killed at 104 or 105 weeks.

L-Tryptophan had little toxic effect on the rats; mean body weight loss was minimal and survival of dosed groups of both sexes was high. In the mice, mean body weights of dosed animals were lower than those of controls throughout most of the bioassay, particularly in the females. Sufficient numbers of rats were at risk to termination of the study for development of late-appearing tumors, and sufficient numbers of mice were at risk beyond 52 weeks of the study for development of tumors.

No neoplasms occurred in a statistically significant incidence among dosed rats when compared with controls.

In both male and female mice, neoplasms of the hematopoietic system occurred at higher incidences in the low-dose groups than in the matched-control groups (males: controls 0/12, low-dose 9/34, high-dose 2/33; females: controls 2/13, low-dose 6/33, high-dose 1/35). These incidences, however, are not statistically significant, using the Bonferroni correction, and therefore, no tumors are considered to be related to the administration of the test chemical.

It is concluded that under the conditions of this bioassay, L-tryptophan was not carcinogenic for Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

L-Tryptophan (CAS 73-22-3; NCI C01729) is an essential amino acid for humans, and a precursor of the neurohormones serotonin (5-hydroxytryptamine) and melatonin (N-acetyl-5-methoxytryptamine), and the B vitamin nicotinic acid (Orten and Neuhaus, 1975). It is found in small concentrations in casein, and in many foods (Stecher, 1968; Food and Agriculture Organization, 1970).

In the 1950's, there were two reports that the dietary administration of DL-tryptophan to rats modified the carcinogenic effects of 2-acetylaminofluorene. The combined administration of these compounds resulted in the production of bladder tumors which were not found in animals dosed with 2-acetylaminofluorene alone (Dunning et al., 1950; Boyland et al., 1954). In subsequent studies in which the tryptophan metabolites indole, indican, or 3-hydroxyanthranilic acid were administered by subcutaneous injection to mice, malignant tumors of the reticuloendothelial system, and leukemia were observed, but there was no evidence of bladder tumors (Ehrhart and Stich, 1957, and 1958; Ehrhart et al., 1959). Tryptophan metabolites were also tested in mice by bladder implantation techniques, and among those that were positive were o-aminophenol derivatives such as 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and 2-amino-3-hydro-

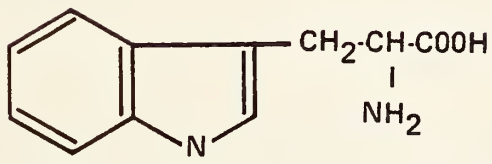
xyacetophenone (Allen et al., 1957; Bryan et al., 1964), although L-tryptophan itself was negative (Boyland et al., 1964). Finally, DL-tryptophan induced bladder hyperplasia but no cancer in dogs when fed in high doses for 1 year (Radomski et al., 1969, 1970, and 1977) which led these authors to the conclusion that tryptophan metabolites may act as co-carcinogens.

L-tryptophan was selected for study in the Carcinogenesis Testing Program because the evidence available at the time of selection suggested that tryptophan or a metabolite was involved in the etiology of bladder cancer.

II. MATERIALS AND METHODS

A. Chemical

L-TRYPTOPHAN



L-Tryptophan (L-α-amino-β-indolepropionic acid) was obtained in a single batch (Lot No. C-8-30-72) for the chronic studies from Carroll Products, Wood River Junction, Rhode Island. The identity and purity of this batch was confirmed in analyses at Midwest Research Institute. No impurities were found by thin-layer chromatography. The melting point was 275-284°C (literature: 278°C, Dictionary of Organic Compounds, 1965). Elemental analyses (C, H, N) were consistent with $C_{11}H_{12}N_2O_2$, the molecular formula of tryptophan. Nuclear magnetic resonance, infrared, and ultraviolet spectra were in agreement with the structure and matched the spectra given in the literature.

The chemical was stored in the original container at 5°C.

B. Dietary Preparation

Test diets were prepared every 2 weeks by mixing a known amount of sifted L-tryptophan with a small amount of Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) in a portable mixer, then adding this mixture to the required amount of animal meal and mixing in a twin-shell blender for 10 minutes. Tests of the concentration or stability of the chemical in feed were not performed.

The prepared diets were stored at room temperature in sealed plastic containers.

C. Animals

For the subchronic studies, male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, and male Swiss mice were obtained from Purina Laboratories, St. Louis, Missouri. All animals were 30 days of age on arrival at the laboratory. They were quarantined for 7 days and then placed on study.

For the chronic studies, Fischer 344 rats and B6C3F1 mice were obtained from Charles River Laboratories under a contract with the Division of Cancer Treatment, National Cancer Institute. On arrival at the laboratory, male and female rats were 30 days of

age, male mice 31 days of age, and female mice 38 days of age. All animals were quarantined for 12 days. Animals with no visible signs of disease were assigned to control or dosed groups and earmarked for individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature range was 20-24°C, and the relative humidity was 40-60%. The room air was changed 15 times per hour and passed through both intake and exhaust fiberglass roughing filters. In addition to natural light, illumination was provided by fluorescent light for 9 hours per day. Food and water were supplied daily and were available ad libitum.

All animals were housed five per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The rat cages were provided with Iso-Dri® hardwood chip bedding (Carworth, Edison, N.J.), and cage tops were covered with disposable filter bonnets; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.) and cage tops were covered with filter bonnets beginning at week 86. Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; and racks were cleaned once per week.

The rats and mice were housed in separate rooms. Control animals were housed with respective dosed animals. Animals administered L-tryptophan were maintained in the same rooms as animals of the same species being dosed with the following chemicals:

RATS

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine hydrochloride) (CAS 136-40-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea (tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)

MICE

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine hydrochloride) (CAS 136-40-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)

N-(p-toluenesulfonyl)-N′-hexamethyleniminourea
(tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate
(phenesterin) (CAS 3546-10-9)

estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate)
(estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride
(MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR)
(CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester)
hydrochloride [IPD] (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione
(ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-
amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine
hydrochloride (phenoxybenzamine hydrochloride) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide
monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses of L-tryptophan, on the basis of which two

different concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. In the subchronic studies, the chemical was administered in feed at concentrations of 1,000, 5,000, 10,000, 25,000, or 50,000 ppm to both male Sprague-Dawley rats and male Swiss mice. Dosed animals received the test diets 7 days per week for 45 days and then were observed for an additional 45 days. Five animals of each species were dosed at each concentration, and 19 rats and 20 mice were used as untreated controls.

There were no deaths at any dose among either the rats or the mice, and final body weights of the dosed animals were within 15% of the controls. The low and high doses for the chronic studies using rats or mice were set at 25,000 and 50,000 ppm to avoid exceeding the limit of 5% of the test compound in feed.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and animals that were moribund were killed and necropsied. Rats and mice were weighed individually every 2 weeks through week 86, and

Table 1. Design of L-Tryptophan Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	L-Tryptophan in Diet[b] (ppm)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Matched-Control	15	0		105
Low-Dose	35	25,000	78	26-27
High-Dose	35	50,000	78	26
Female				
Matched-Control	15	0		105
Low-Dose	35	25,000	78	26-27
High-Dose	35	50,000	78	26-27

[a]All animals were 42 days of age when placed on study.

[b]The dosed animals were fed test diets 5 days per week and control diets 2 days per week.

Table 2. Design of L-Tryptophan Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	L-Tryptophan in Diet[b] (ppm)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Matched-Control	15	0		104
Low-Dose	35	25,000	78	26
High-Dose	35	50,000	78	26
Female				
Matched-Control	15	0		104
Low-Dose	35	25,000	78	26
High-Dose	35	50,000	78	26

[a]Male mice were 43 days of age and female mice were 50 days of age when placed on study.

[b]The dosed animals were fed test diets 5 days per week and control diets 2 days per week. Test diets were withdrawn for 17 consecutive days during weeks 65 to 67, due to continued low weight gains.

once every month for the remainder of the study. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, muscle, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Peripheral blood smears were prepared from each animal whenever possible. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals from which particular organs or tissues

were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for

a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for a number of dosed groups (k) are

compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which

the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a dosed group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control

group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of the low- and high-dose male rats were lower than those of the male matched controls, while body weights of the female rats were similar to those of the female matched controls. Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to wide variation. No other chemical-related signs of toxicity in the dosed animals were recorded (figure 1).

To control respiratory disease, rats received oxytetracycline in the drinking water at 0.6 mg/ml during weeks 39 to 43 and at 0.3 mg/ml during weeks 43 to 44.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed L-tryptophan in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2.

In each sex, the Tarone test result for positive dose-related trend in mortality is not significant. In male rats, 28/35 (80%) of the high-dose group, 27/35 (77%) of the low-dose group, and

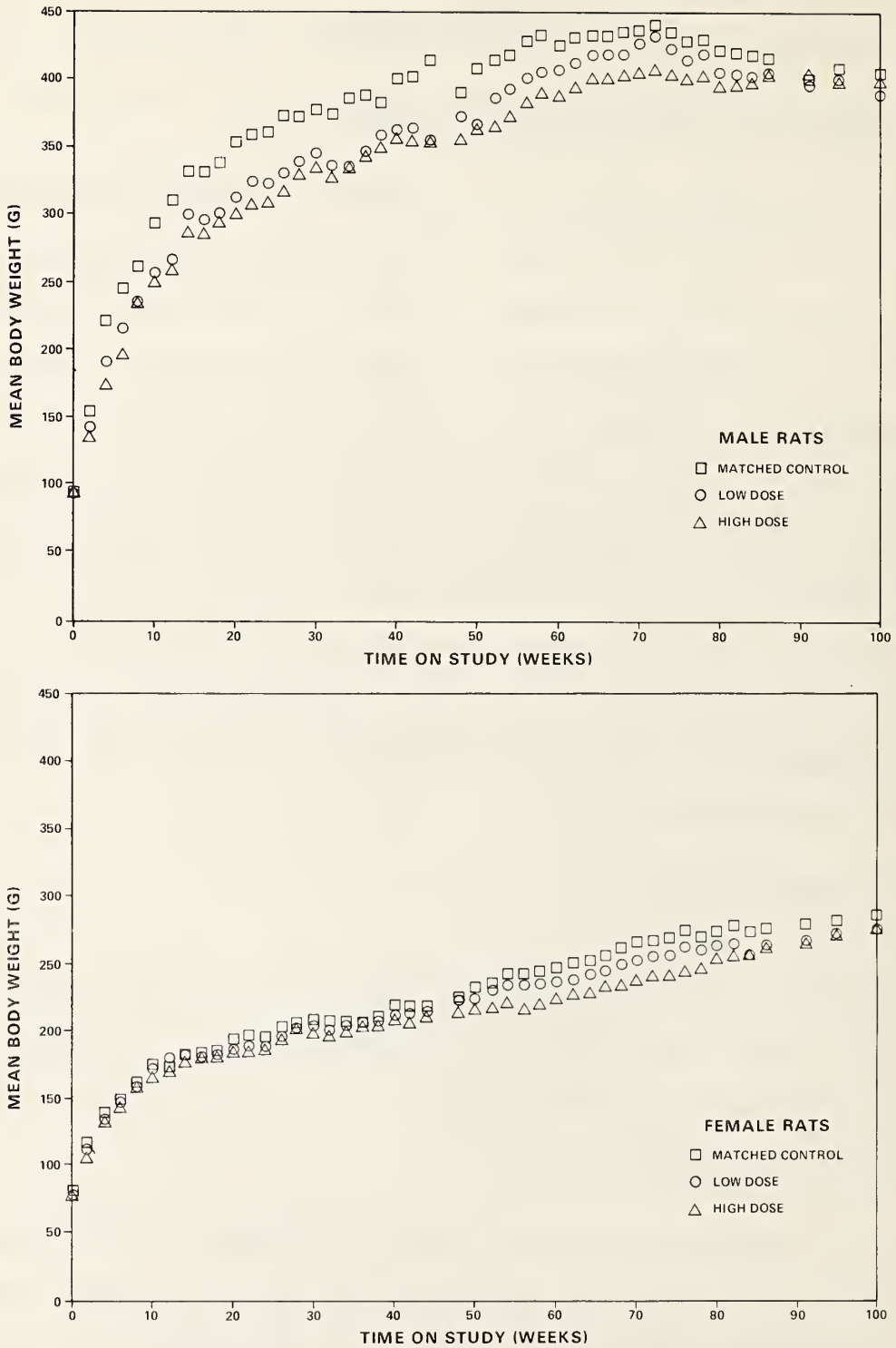


Figure 1. Growth Curves for Rats Fed L-Tryptophan in the Diet

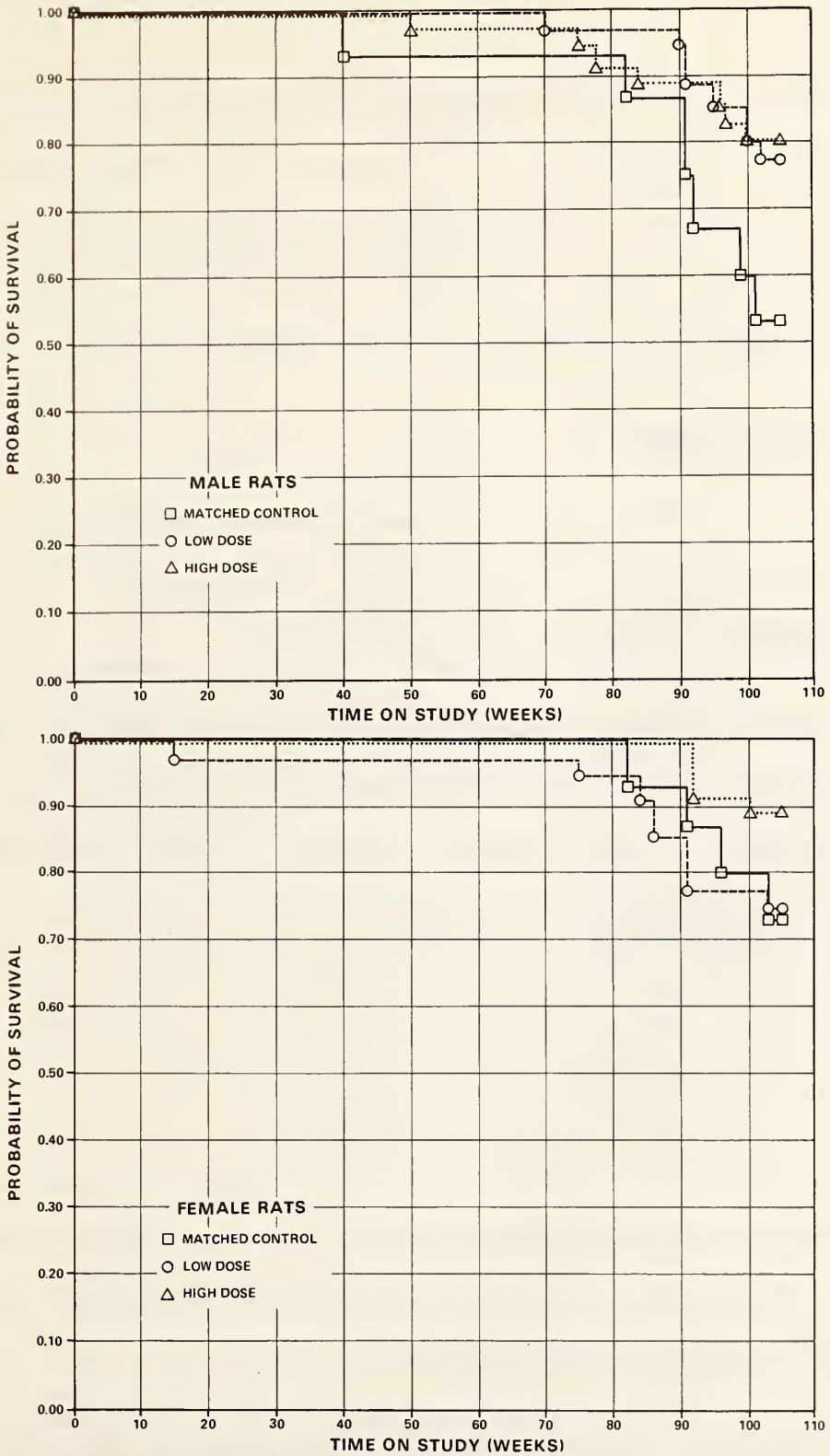


Figure 2. Survival Curves for Rats Fed L-Tryptophan in the Diet

8/15 (53%) of the matched controls lived to the end of the study. In females, 31/35 (89%) of the high-dose group, 26/35 (74%) of the low-dose group, and 11/15 (73%) of the matched controls lived to the end of the study. Sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, tables C1 and C2.

A variety of neoplasms occurred in both the matched-control and dosed groups. Some types of neoplasms occurred only in rats of dosed groups, or with a greater frequency in dosed groups when compared with controls. Fibromas of the subcutaneous tissue occurred in 4/34 low-dose and 3/34 high-dose male rats, but in 0/15 controls. These lesions, however, are not uncommon in this strain of rat independent of any treatment.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the control and dosed groups. These nonneoplastic lesions are commonly seen in aged rats.

In the judgment of the pathologists, L-tryptophan was not carcino-

genic when fed to Fischer 344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that were observed in at least two animals in one group and with an incidence of at least 5% in one or more than one group.

In each sex, the results of the Cochran-Armitage test for positive dose-related trend and of the Fisher exact test for direct comparison of incidences between the matched-control group and each of the dosed groups in the positive direction are not significant.

In male rats, the Cochran-Armitage test results indicate a linear trend in the negative direction in the incidences of leukemia ($P = 0.014$), C-cell adenomas or carcinomas of the thyroid ($P = 0.004$), and interstitial-cell tumors of the testis ($P = 0.016$). The probability level of the Fisher exact test of the incidence of C-cell adenomas or carcinomas of the thyroid in male rats is 0.007, reflecting the higher incidence in the control group (5/14, 36%) than in the high-dose group (1/32, 3%). These significant results in the negative direction cannot be explained by differential survival. In female rats, the Cochran-Armitage

test results of the incidence of fibroadenomas in the mammary gland also indicate a significant trend (P = 0.027) in the negative direction.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one or less than one is included, indicating the absence of positive significant results. It should also be noted that most of the intervals have upper limits greater than one, indicating the theoretical possibility of the induction of tumors by L-tryptophan, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of both low- and high-dose mice of each sex were lower than those of the matched controls, becoming increasingly lower from week 20 through week 78 of administration of the chemical (figure 3). The body weights of the dosed groups approached those of the controls after L-tryptophan feeding was discontinued, especially in the males. Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to wide variation. There were no other clinical signs of chemical-related toxicity.

To control respiratory disease, mice received oxytetracycline in the drinking water at 0.6 mg/ml during week 66 and at 0.3 mg/ml during week 67. Propylene glycol was vaporized in the mouse room during weeks 66 to 76 to decrease the transmission of micro-organisms that may have caused the respiratory disease.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed L-tryptophan in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4.

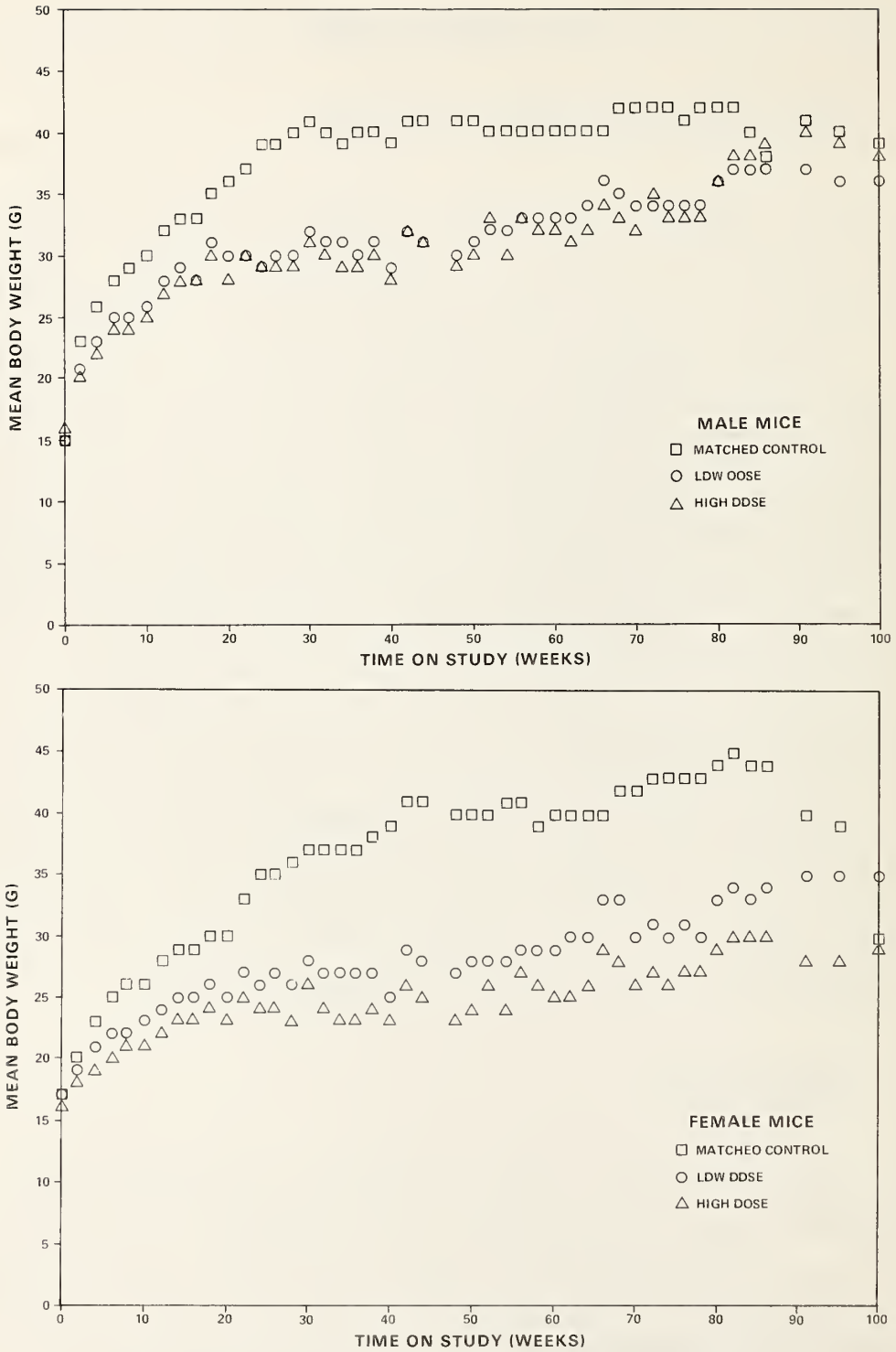
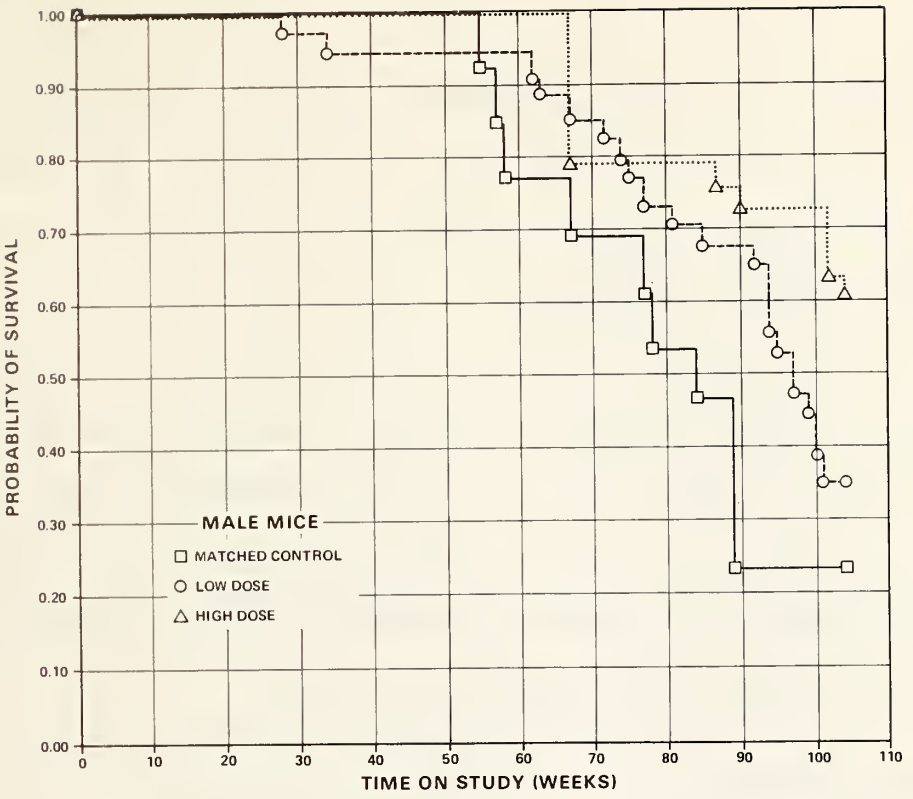


Figure 3. Growth Curves for Mice Fed L-Tryptophan in the Diet



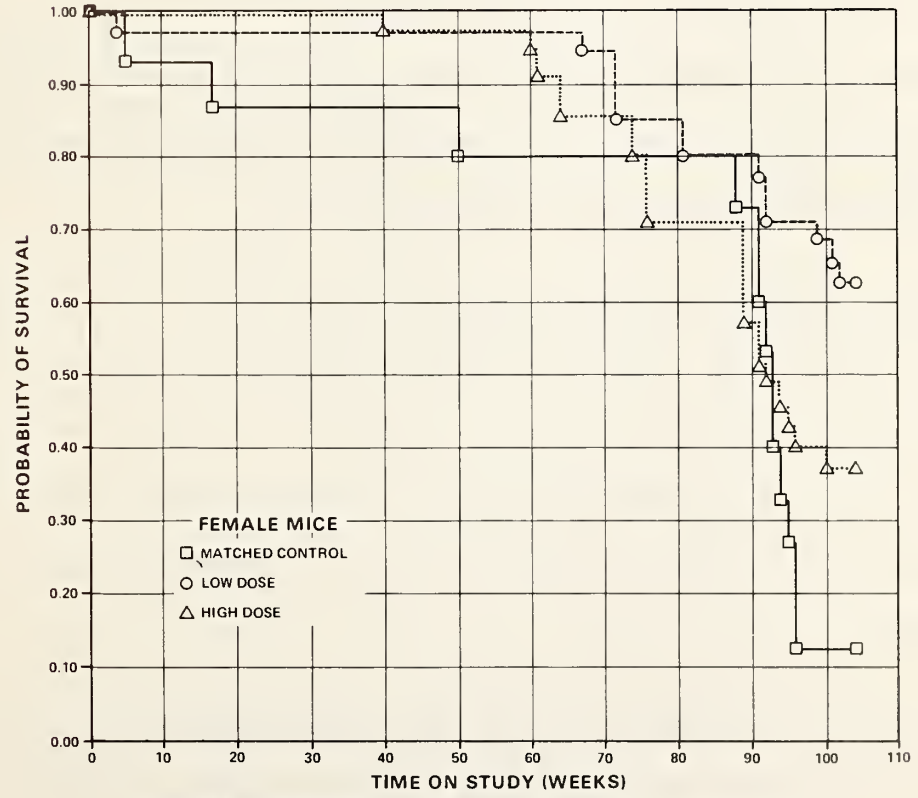


Figure 4. Survival Curves for Mice Fed L-Trytophan in the Diet

In each sex, the Tarone test result for positive dose-related trend in mortality is not significant. In male mice, 21/33 (64%) of the high-dose group, 12/35 (34%) of the low-dose group, and 3/15 (20%) of the matched controls were alive at the last week (104) of the study. In females, 13/35 (37%) of the high-dose group, 22/35 (63%) of the low-dose group, and 2/15 (13%) of the matched-control group lived to the end of the study. More than 50% of the mice in each group of either sex studied lived beyond week 75 on study, providing sufficient numbers of animals at risk for the development of late-appearing tumors.

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, tables B1 and B2; findings on nonneoplastic lesions are summarized in Appendix D, tables D1 and D2.

With the exception of the lymphatic tumors and a few hematopoietic tumors, the neoplasms listed in Appendix B appeared with approximately equal frequency in control and dosed mice or appeared in insignificant numbers. These lesions are not uncommon in the B6C3F1 strain of mouse independent of any treatment.

The incidence of hematopoietic neoplasms was higher in the dosed than in the matched-control groups, with the highest incidence in

the low-dose groups. The incidences of these lesions were as follows:

	Matched Control	Low Dose	High Dose
MALES			
Number of Mice Necropsied	(12)	(34)	(33)
Brain: Malignant lymphoma, histiocytic type	0	4	0
Multiple organs, lymphoreticular: Malignant lymphoma, histiocytic or mixed type	0	1	1
Spleen: Malignant lymphoma, lymphocytic type	0	0	1
Mandibular lymph node: Malignant lymphoma, lymphocytic type	0	1	0
Mesenteric lymph node: Malignant lymphoma, lymphocytic or histiocytic type	0	2	0
Liver: Malignant lymphoma, histiocytic type	0	1	0
Total incidence of mice with tumors (%)	0/12(0)	9/34(26)	2/33(6)

	Matched Control	Low Dose	High Dose
FEMALES			
Number of Mice Necropsied	(13)	(33)	(35)
Brain: Malignant lymphoma, histiocytic type	0	0	1
Multiple organs, lymphoreticular and hematopoietic: Malignant lymphoma, lymphocytic or histiocytic type	0	5	0
lymphocytic leukemia	0	1	0
Mesenteric lymph node: Malignant lymphoma, histiocytic type	1	0	0
Peyer's patches: Malignant lymphoma, histiocytic type	1	0	0
Total incidence of mice with tumors (%)	2/13(15)	6/33(18)	1/35(3)

Malignant lymphomas consisted of three cell types. (1) The lymphocytic type was comprised of cells having a small, darkly basophilic to large, lightly basophilic vesicular nucleus and a rim of eosinophilic cytoplasm. (2) The histiocytic type was comprised of cells with a large, open-faced vesicular nucleus and a distinct eosinophilic nucleolus. (3) The mixed type was a combination of the lymphocytic and histiocytic types of cells.

The malignant lymphomas were observed to be either generalized, involving several organs, or solitary, involving only one organ. The generalized lymphomas always involved the spleen, liver, and one or more lymph nodes. The solitary lymphomas involved the

spleen, liver, mandibular lymph nodes, mesenteric lymph nodes, Peyer's patches or brain. The brain lesions usually involved the meninges and choroid plexuses with variable degrees of perivascular cuffing.

Neoplastic cells having a small, darkly basophilic nucleus and minimal cytoplasm were the predominant type of cell in lymphocytic leukemia. The lymphocytic infiltration within the liver was diffuse when compared with the more solid arrangement of the cells during lymphoma. A large area of hemorrhage within the brain was another feature of the leukemia.

A mast-cell sarcoma involving the wall of the stomach, liver, and mesentery was observed in a low-dose female. The neoplastic mast cells had a large, basophilic nucleus and an abundant cytoplasm engorged with basophilic granules.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the control and dosed groups (Appendix D). These nonneoplastic lesions are commonly seen in aged mice; however, the suppurative lesions involving the trachea and lungs were associated with early deaths. The decreased life spans, especially prominent in the low-dose males, high-dose females,

and both control groups, may have resulted in a reduced incidence of tumors in several of these groups.

The incidence of lymphoreticular and other neoplasms of the hematopoietic system was higher in both male and female low-dose groups of mice fed L-tryptophan than in either the matched-control or high-dose mice of either sex.

In the judgment of the pathologists, L-tryptophan may be associated with the increased incidence of lymphoreticular neoplasms in low-dose male and female B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that were observed in at least two animals in one group and with an incidence of at least 5% in one or more than one group.

In male mice, when the incidence of lymphomas in the low-dose group is compared with that in the control group, there is a higher proportion in the low-dose group of male mice ($P = 0.048$), but this probability level is above that of 0.025 required for significance using the Bonferroni inequality for multiple comparisons. The incidences of this tumor in the high-dose group

of male mice and in both dosed groups of female mice are not statistically significant when the Fisher exact test is applied.

In each sex, the results of the Cochran-Armitage test for positive dose-related trend and of the Fisher exact test for direct comparison of the incidence in the matched-control group with the incidences in each of the dosed groups are not significant for any of the tumors.

In each of the 95% confidence intervals of relative risk, except for the occurrence of lymphoma in low-dose male mice, the value of one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by L-tryptophan, which could not be detected under the conditions of this test.

V. DISCUSSION

There was little evidence that the administration of L-tryptophan was toxic to rats. Differences in mean body weight among dosed and control groups were minimal, and survival of the dosed groups of both sexes was high. Sufficient numbers of rats were at risk for development of late-appearing tumors.

In the mice, however, the administration of L-tryptophan resulted in lowered mean body weights throughout most of the study, particularly in the females. Survival rates were lower in low-dose males, high-dose females, and corresponding matched controls than in high-dose males and low-dose females. More than 50% of the mice in each group lived to week 52 or longer; thus, sufficient numbers of mice were at risk for development of tumors appearing up to that time.

No neoplasms occurred in a statistically significant incidence among the dosed rats when compared with the control rats.

In both male and female mice, neoplasms of the hematopoietic systems (all sites) occurred at higher incidences in the low-dose groups than in the matched-control groups (males: controls 0/12, low-dose 9/34, high-dose 2/33; females: controls 2/13, low-dose 6/33, high-dose 1/35); the incidence in females was not significant, and in males the probability of $P = 0.048$ was not

significant using the Bonferroni correction. Because of the lack of statistical significance and because of the known variability of the incidence of these tumors in B6C3F1 mice, they are not considered to be related to administration of the chemical.

In previous studies, malignant reticuloendothelial tumors and leukemia were reported in mice administered various metabolites of tryptophan by oral or subcutaneous routes (Ehrhart and Stich, 1957 and 1958; Ehrhart et al., 1959; Rauschenbach et al., 1963; Rauschenbach et al., 1966). In addition, numerous studies have been conducted to determine the effect of tryptophan on the carcinogenicity of other compounds. Dunning et al. (1950) and Kawachi et al. (1968) found that tryptophan increased or extended the carcinogenicity of known carcinogens, while Okajima et al. (1971), Oyasu et al. (1972), and Evarts and Brown (1977) demonstrated that tryptophan decreased the incidence of liver tumors induced by known carcinogens.

It is concluded that under the conditions of this bioassay, L-tryptophan was not carcinogenic for Fischer 344 rats or B6C3F1 mice.

VI. BIBLIOGRAPHY

Allen, M. J., Boyland, E., Dukes, C. E., Horning, E. S., and Watson, J. G., Cancer of the urinary bladder induced in mice with metabolites of aromatic amines and trytophan. Brit. J. Cancer 11:212-228, 1957.

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

Berenblum, I., ed., Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of the UICC, Vol. 2, International Union Against Cancer, Geneva, 1969.

Boyland, E., Harris, J., and Horning, E. S., The induction of carcinoma of the bladder in rats with acetamidofluorene. Brit. J. Cancer 8:647-654, 1954.

Bryan, G. T., Brown, R. R., and Price, J. M., Mouse bladder carcinogenicity of certain tryptophan metabolites and other aromatic nitrogen compounds suspended in cholesterol. Cancer Res. 24:596-602, 1964.

Cox, D. R., Regression models and life tables. J. R. Statist. Soc. B 34 (2):187-220, 1972.

Cox. D. R., Analysis of Binary Data, Methuen & Co., Ltd., London, 1970, pp. 48-52.

Dictionary of Organic Compounds, Vol. 5, Oxford University Press, New York, 1965, p. 3211.

Dunning, W. F., Curtis, M. R., and Maun, M. E., The effect of added dietary tryptophane on the occurrence of 2-acetylamino-fluorene-induced liver and bladder cancer in rats. Cancer Res. 10(7):454-459, 1950.

Ehrhart, H., Georgii, A., and Stanislawksi, K. Ueber die leukämogene Wirkung von 3-Hydroxy-anthranilsäure bei RFH-Mäusen. Klinische Wochenschrift 37(20):1054-1059, 1959.

Ehrhart, H. and Stich, W., Leukamieerzeugung bei Mäusen durch Injektion wässriger Indicanlosung. Klinische Wochenschrift 36(22):1056-1061, 1958.

Ehrhart, H. and Stich, W., Die Indol-Leukämie bei der weissen Maus. Klinische Wochenschrift 35(10):504-511, 1957.

Evarts, R. P. and Brown, C. A., Effect of L-tryptophan on diethylnitrosamine and 3'-methyl-4-N-dimethylaminoazobenzene hepatocarcinogenesis. Fd. Cosmet. Toxicol. 15:431-435, 1977.

Food and Agriculture Organization of the United Nations, Amino-Acid Content of Foods and Biological Data on Proteins, No. 24, Food and Agriculture Organization, Rome, 1970.

Gart J. J. The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification. Rev. Int. Stat. Inst. 39 (2):148-169, 1971.

Kaplan, E. L. and Meier, P., Nonparametric estimation from incomplete observations. J. Am. Statist. Assoc. 53:457-481, 1958.

Kawachi, T., Hirata, Y., and Sugimura, T., Enhancement of N-nitrosodiethylamine hepatocarcinogenesis by L-tryptophan in rats. GANN 59:523-525, 1968.

Linhart, M. S., Cooper, J. A., Martin, R. L., Page, N. P., and Peters, J. A., Carcinogenesis bioassay data system. Comp. and Biomed. Res. 7:230-248, 1974.

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill Book Co., New York, 1966, pp. 6-10.

Okajima, E., Hiramatsu, T., Motomiya, Y., Iriya, K., Ijuin, M., and Ito, N., Effect of DL-tryptophan on tumorigenesis in the urinary bladder and liver of rats treated with N-nitrosodibutylamine. GANN 62:163-169, 1971.

Orten, J. M. and Neuhaus, O. W., Basic principles of biochemistry. Human Biochemistry, The C. V. Mosby Co., St. Louis, 1975, pp. 332-336.

Oyasu, R., Kitajima, T., Hopp, M., and Sumie, H., Enhancement of urinary bladder tumorigenesis in hamsters by coadministration of 2-acetylaminofluorene and indole. Cancer Res. 32:2027-2033, 1972.

Radomski, J. L., Radomski, T., and MacDonald, W. E., Cocarcinogenic interaction between D,L-tryptophan and 4-aminobiphenyl or 2-naphthylamine in dogs. J. Natl. Cancer Inst. 58(6):1831-1834, 1977.

Radomski, J. L., Deichmann, W. B., and Brill, E., Tryptophan as a co-carcinogen in bladder cancer. Proc. Am. Assoc. Cancer Res. 10:71, 1969.

Radomski, J. L., Deichmann, W. B., and Brill, E., Transitional cell hyperplasia in the bladders of dogs fed trytophan. Proc. Am. Assoc. Cancer Res. 11:65, 1970.

Rauschenbach, M. O., Zharova, E. I., Ivanova, W. D., Kuznezova, L. E., Miasitcheva, N. W., and Sergeev, A. V., The investigation of the mechanisms of leucomogenic action of the tryptophan metabolites. Ninth International Cancer Congress, Abstracts, Cancer Institute, Tokyo, 1966, p. 194.

Rauschenbach, M. O., Jarova, E. I., and Protasova, T. C., Blastomogenic properties of certain metabolites of tryptophane. Acta-Unio Internationalis Contra Cancrum 19:660-662, 1963.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G., Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo (a) pyrene and ferric oxide. Cancer Res. 32:1073-1081, 1972.

Stecher, P. G., ed., The Merck Index, 8th ed., Merck & Co., Inc. Rahway, N. J., 1968, pp. 216 and 1086.

Tarone, R. E., Tests for trend in life table analysis. Biometrika 62 (3):679-682, 1975.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED L-TRYPTOPHAN IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED L-TRYPTOPHAN IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	34	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	34	34
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(34)	(34)
SQUAMOUS CELL PAPILLOMA		1 (3%)	
SQUAMOUS CELL CARCINOMA	1 (7%)		
*SUBCUT TISSUE	(15)	(34)	(34)
FIBROMA		4 (12%)	3 (9%)
LIPOMA		1 (3%)	1 (3%)
NEUROFIBROSARCOMA			1 (3%)
RESPIRATORY SYSTEM			
*LUNG	(15)	(34)	(34)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (3%)	1 (3%)
NEUROFIBROSARCOMA, METASTATIC			1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(34)	(34)
LEUKEMIA,NOS			1 (3%)
UNDIFFERENTIATED LEUKEMIA	4 (27%)	2 (6%)	
LYMPHOCYTIC LEUKEMIA		1 (3%)	
#MANDIBULAR L. NODE	(2)	(4)	(9)
NEUROFIBROSARCOMA, METASTATIC			1 (11%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(15)	(34)	(34)
FIBROMA	1 (7%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#COLON	(15)	(34)	(34)
ADENOMATOUS POLYP, NOS			1 (3%)
MUCINOUS ADENOCARCINOMA			1 (3%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(11)	(30)	(30)
CHROMOPHOBE ADENOMA	1 (9%)	2 (7%)	5 (17%)
CRANIOPHARYNGIOMA		1 (3%)	
#ADRENAL	(15)	(34)	(34)
PHEOCHROMOCYTOMA		1 (3%)	
GANGLIONEUROMA			1 (3%)
#THYROID	(14)	(33)	(32)
FOLLICULAR-CELL CARCINOMA		2 (6%)	
C-CELL ADENOMA	4 (29%)	2 (6%)	1 (3%)
C-CELL CARCINOMA	1 (7%)	4 (12%)	
MUCINOUS ADENOCARCINOMA		1 (3%)	
#PANCREATIC ISLETS	(15)	(33)	(34)
ISLET-CELL ADENOMA		2 (6%)	
ISLET-CELL CARCINOMA			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(34)	(34)
FIBROADENOMA	2 (13%)		
#TESTIS	(15)	(34)	(33)
INTERSTITIAL-CELL TUMOR	13 (87%)	31 (91%)	21 (64%)
NERVOUS SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(15)	(34)	(34)
ADENOMA, NOS			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(15)	(34)	(34)
LIPOMA			1 (3%)
MESOTHELIOMA BENIGN		2 (6%)	1 (3%)
*PLEURA	(15)	(34)	(34)
ALVEOLAR/BRONCHIOLAR CA, METASTA			1 (3%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	2	3	4
MORIBUND SACRIFICE	5	5	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	8	27	28
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	32	29
TOTAL PRIMARY TUMORS	28	58	41
TOTAL ANIMALS WITH BENIGN TUMORS	14	32	26
TOTAL BENIGN TUMORS	22	46	36
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	10	5
TOTAL MALIGNANT TUMORS	6	11	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			2
TOTAL SECONDARY TUMORS			3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED L-TRYPTOPHAN IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	35	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	34
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(14)	(35)	(34)
LIPOMA		1 (3%)	
RESPIRATORY SYSTEM			
*LUNG	(14)	(35)	(34)
ADENOCARCINOMA, NOS, METASTATIC		1 (3%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(14)	(35)	(34)
UNDIFFERENTIATED LEUKEMIA	1 (7%)		
*MEDIASTINAL L.NODE	(3)	(5)	(2)
ADENOCARCINOMA, NOS, METASTATIC		1 (20%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(12)	(27)	(32)
CHROMOPHOBE ADENOMA	4 (33%)	12 (44%)	9 (28%)
CHROMOPHOBE CARCINOMA		1 (4%)	2 (6%)
#ADRENAL	(14)	(35)	(34)
CORTICAL ADENOMA			1 (3%)
PHEOCHROMOCYTOMA	1 (7%)		
PHEOCHROMOCYTOMA, MALIGNANT			1 (3%)
#THYROID	(14)	(32)	(33)
FOLLICULAR-CELL CARCINOMA		1 (3%)	
C-CELL ADENOMA	1 (7%)	2 (6%)	1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(14)	(35)	(34)
ADENOMA, NOS			2 (6%)
ADENOCARCINOMA, NOS			1 (3%)
FIBROADENOMA	4 (29%)	7 (20%)	2 (6%)
#UTERUS	(13)	(35)	(33)
ADENOCARCINOMA, NOS		1 (3%)	1 (3%)
SARCOMA, NOS		1 (3%)	
ENDOMETRIAL STROMAL POLYP	3 (23%)	3 (9%)	3 (9%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(14)	(35)	(34)
ADENOCARCINOMA, NOS, METASTATIC		1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	3	2	1
MORIBUND SACRIFICE	1	7	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	11	26	31
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	24	19
TOTAL PRIMARY TUMORS	15	29	23
TOTAL ANIMALS WITH BENIGN TUMORS	11	21	15
TOTAL BENIGN TUMORS	14	25	18
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	4	5
TOTAL MALIGNANT TUMORS	1	4	5
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED L-TRYPTOPHAN IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED L-TRYPTOPHAN IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	@35
ANIMALS NECROPSIED	12	34	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	34	33
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(12)	(34)	(33)
FIBROSARCOMA		1 (3%)	1 (3%)
RESPIRATORY SYSTEM			
#LUNG	(12)	(33)	(32)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (9%)	2 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (3%)	
HEMATOPOIETIC SYSTEM			
#BRAIN	(11)	(32)	(32)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		4 (13%)	
*MULTIPLE ORGANS	(12)	(34)	(33)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (3%)	
#SPLEEN	(11)	(32)	(32)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)
#MANDIBULAR L. NODE	(4)	(14)	(3)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (7%)	
#MESENTERIC L. NODE	(4)	(14)	(3)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (7%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (7%)	
#LIVER	(12)	(34)	(33)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
CIRCULATORY SYSTEM			
NONE			

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

@ 35 ANIMALS WERE INITIALLY IN THE STUDY, BUT TWO AIMALS WERE FOUND TO BE FEMALES IN A MALE GROUP.

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(12)	(34)	(33)
HEPATOCELLULAR ADENOMA	1 (8%)	4 (12%)	5 (15%)
HEPATOCELLULAR CARCINOMA		1 (3%)	2 (6%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(11)	(31)	(28)
FOLLICULAR-CELL ADENOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
*TRIGEMINAL GANGLION	(12)	(34)	(33)
NEURILEMOMA, MALIGNANT		2 (6%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	5	9	8
MORIBUND SACRIFICE	5	13	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED	2	1	
TERMINAL SACRIFICE	3	12	20
ANIMAL MISSING			
ANIMAL DELETED(WRONG SEX)			2
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	15	12
TOTAL PRIMARY TUMORS	1	22	12
TOTAL ANIMALS WITH BENIGN TUMORS	1	7	7
TOTAL BENIGN TUMORS	1	8	7
TOTAL ANIMALS WITH MALIGNANT TUMORS		12	5
TOTAL MALIGNANT TUMORS		14	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED L-TRYPTOPHAN IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	13	33	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	13	33	35
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(13)	(33)	(35)
SARCOMA, NOS		1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(13)	(32)	(35)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (8%)		1 (3%)
HEMATOPOIETIC SYSTEM			
#BRAIN	(12)	(33)	(35)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)
*MULTIPLE ORGANS	(13)	(33)	(35)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (3%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		4 (12%)	
MAST-CELL SARCOMA		1 (3%)	
LYMPHOCYTIC LEUKEMIA		1 (3%)	
#MESENTERIC L. NODE	(4)	(17)	(15)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (25%)		
#PEYERS PATCH	(13)	(33)	(35)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (8%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(13)	(32)	(35)
HEPATOCELLULAR ADENOMA		1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA			1 (3%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(5)	(22)	(28)
CHROMOPHOBE ADENOMA	1 (20%)	1 (5%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(13)	(32)	(34)
ENDOMETRIAL STROMAL POLYP		1 (3%)	
NERVOUS SYSTEM			
*TRIGEMINAL GANGLION	(13)	(33)	(35)
NEURILEMOMA, MALIGNANT		1 (3%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(13)	(33)	(35)
SARCOMA, NOS		1 (3%)	
ALL OTHER SYSTEMS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	3	7	6
MORIBUND SACRIFICE	10	6	16
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	2	22	13
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	11	3
TOTAL PRIMARY TUMORS	4	13	3
TOTAL ANIMALS WITH BENIGN TUMORS	2	3	1
TOTAL BENIGN TUMORS	2	3	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	8	2
TOTAL MALIGNANT TUMORS	2	10	2
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN RATS FED L-TRYPTOPHAN IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED L-TRYPTOPHAN IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	34	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	34	34
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(34)	(34)
EPIDERMAL INCLUSION CYST	1 (7%)	1 (3%)	2 (6%)
INFLAMMATION, FOCAL			1 (3%)
INFLAMMATION, NECROTIZING			1 (3%)
*SUBCUT TISSUE	(15)	(34)	(34)
HEMORRHAGE			1 (3%)
HEMORRHAGIC CYST		1 (3%)	
HEMORRHAGE, CHRONIC			1 (3%)
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(33)	(34)
INFLAMMATION, SUPPURATIVE	5 (36%)	8 (24%)	8 (24%)
PLASMA-CELL INFILTRATE			1 (3%)
#LUNG	(15)	(34)	(34)
PNEUMONIA INTERSTITIAL CHRONIC		1 (3%)	
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (7%)	1 (3%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (3%)
METAPLASIA, SQUAMOUS		1 (3%)	
#LUNG/ALVEOLI	(15)	(34)	(34)
EMPHYSEMA, NOS		1 (3%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(15)	(34)	(31)
ATROPHY, NOS	2 (13%)	7 (21%)	11 (35%)
#SPLEEN	(15)	(34)	(34)
HYPERPLASIA, LYMPHOID			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIESIS	2 (13%)	2 (6%)	
#MANDIBULAR L. NODE	(2)	(4)	(9)
HYPERPLASIA, PLASMA CELL		1 (25%)	
#MESENTERIC L. NODE	(2)	(4)	(9)
HYPERPLASIA, LYMPHOID		1 (25%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(15)	(34)	(34)
CALCIFICATION, DYSTROPHIC	1 (7%)		
DIGESTIVE SYSTEM			
#LIVER	(15)	(34)	(34)
INFLAMMATION, NECROTIZING			1 (3%)
HYPERPLASIA, NODULAR	1 (7%)	2 (6%)	1 (3%)
HEMATOPOIESIS	2 (13%)	1 (3%)	
#LIVER/CENTRILOBULAR	(15)	(34)	(34)
NECROSIS, COAGULATIVE	1 (7%)		
#PANCREAS	(15)	(33)	(34)
ATROPHY, NOS		1 (3%)	
#PANCREATIC ACINUS	(15)	(33)	(34)
ATROPHY, NOS		2 (6%)	1 (3%)
ATROPHY, FOCAL			1 (3%)
#PEYERS PATCH	(15)	(34)	(34)
HYPERPLASIA, LYMPHOID		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(15)	(34)	(34)
INFLAMMATION, CHRONIC	14 (93%)	30 (88%)	31 (91%)
ENDOCRINE SYSTEM			
#THYROID	(14)	(33)	(32)
HYPERPLASIA, C-CELL		2 (6%)	1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(34)	(34)
CYST, NOS		1 (3%)	1 (3%)
*PREPUTIAL GLAND	(15)	(34)	(34)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
HYPERKERATOSIS		1 (3%)	
*PROSTATE	(15)	(33)	(34)
INFLAMMATION, SUPPURATIVE		1 (3%)	1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV			2 (6%)
*TESTIS	(15)	(34)	(33)
ATROPHY, NOS			2 (6%)
NERVOUS SYSTEM			
#BRAIN	(15)	(34)	(34)
HEMORRHAGE	3 (20%)		
MALACIA	1 (7%)		
SPECIAL SENSE ORGANS			
*EYE	(15)	(34)	(34)
PUS	1 (7%)		
*EYE/CORNEA	(15)	(34)	(34)
INFLAMMATION, CHRONIC			1 (3%)
*EYE/CRYSTALLINE LENS	(15)	(34)	(34)
MINERALIZATION		1 (3%)	1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(15)	(34)	(34)
NECROSIS, FAT		1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
AUTOLYSIS/NO NECROPSY		1	1

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED L-TRYPTOPHAN IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	35	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	34
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*NASAL CAVITY	(14)	(35)	(34)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
#TRACHEA	(14)	(34)	(34)
INFLAMMATION, SUPPURATIVE	4 (29%)	8 (24%)	10 (29%)
#LUNG	(14)	(35)	(34)
BRONCHOPNEUMONIA SUPPURATIVE	1 (7%)		
BRONCHOPNEUMONIA CHRONIC SUPPURA		1 (3%)	1 (3%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(12)	(31)	(33)
ATROPHY, NOS	7 (58%)	19 (61%)	23 (70%)
#SPLEEN	(14)	(35)	(34)
NECROSIS, COAGULATIVE		1 (3%)	
HEMATOPOIESIS		4 (11%)	1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(14)	(35)	(34)
INFLAMMATION, INTERSTITIAL		1 (3%)	
DIGESTIVE SYSTEM			
#LIVER	(14)	(35)	(34)
HEMORRHAGE	1 (7%)		

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CYTOPLASMIC VACUOLIZATION		1 (3%)	
FOCAL CELLULAR CHANGE			1 (3%)
HYPERPLASIA, NODULAR			1 (3%)
HEMATOPOIESIS		1 (3%)	
#PANCREATIC ACINUS	(14)	(35)	(34)
ATROPHY, NOS		1 (3%)	1 (3%)
ATROPHY, FOCAL			1 (3%)
URINARY SYSTEM			
#KIDNEY	(14)	(35)	(34)
MINERALIZATION		1 (3%)	
PYELONEPHRITIS SUPPURATIVE		1 (3%)	
INFLAMMATION, CHRONIC	10 (71%)	19 (54%)	24 (71%)
NECROSIS, MEDULLARY		1 (3%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(14)	(35)	(34)
CYST, NOS	6 (43%)	1 (3%)	1 (3%)
#UTERUS	(13)	(35)	(33)
DECIDUAL ALTERATION, NOS			1 (3%)
#CERVIX UTERI	(13)	(35)	(33)
CYST, NOS	1 (8%)		
#UTERUS/ENDOMETRIUM	(13)	(35)	(33)
INFLAMMATION, SUPPURATIVE	3 (23%)	7 (20%)	4 (12%)
HYPERPLASIA, CYSTIC	1 (8%)	4 (11%)	3 (9%)
#OVARY/OVIDUCT	(13)	(35)	(33)
INFLAMMATION, SUPPURATIVE			1 (3%)
#OVARY	(13)	(32)	(33)
CYST, NOS	9 (69%)	14 (44%)	11 (33%)
INFLAMMATION, SUPPURATIVE			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC SUPPURATIV		2 (6%)	
NERVOUS SYSTEM			
*SPINAL CORD	(14)	(35)	(34)
DEGENERATION, NOS			1 (3%)
SPECIAL SENSE ORGANS			
*EYE	(14)	(35)	(34)
ATROPHY, NOS		2 (6%)	
*EYE/CRYSTALLINE LENS	(14)	(35)	(34)
MINERALIZATION		2 (6%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(14)	(35)	(34)
STEATITIS			1 (3%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		
AUTOLYSIS/NO NECROPSY	1		1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN MICE FED L-TRYPTOPHAN IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED L-TRYPTOPHAN IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	@35
ANIMALS NECROPSIED	12	34	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	34	33
INTEGUMENTARY SYSTEM			
*SKIN	(12)	(34)	(33)
INFLAMMATION, CHRONIC	1 (8%)		
*SUBCUT TISSUE	(12)	(34)	(33)
HEMORRHAGE			1 (3%)
RESPIRATORY SYSTEM			
#TRACHEA	(12)	(34)	(33)
INFLAMMATION, SUPPURATIVE	2 (17%)	5 (15%)	1 (3%)
#LUNG/BRONCHUS	(12)	(33)	(32)
HYPERPLASIA, LYMPHOID			1 (3%)
#LUNG	(12)	(33)	(32)
BRONCHOPNEUMONIA SUPPURATIVE	3 (25%)	9 (27%)	4 (13%)
BRONCHOPNEUMONIA CHRONIC SUPPURA			1 (3%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (8%)	1 (3%)	2 (6%)
HYPERPLASIA, PLASMA CELL		1 (3%)	1 (3%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(12)	(34)	(33)
ATROPHY, NOS	1 (8%)	2 (6%)	
#SPLEEN	(11)	(32)	(32)
HEMATOPOIESIS		1 (3%)	1 (3%)
#MANDIBULAR L. NODE	(4)	(14)	(3)
HYPERPLASIA, LYMPHOID		1 (7%)	
#MESENTERIC L. NODE	(4)	(14)	(3)
HYPERPLASIA, LYMPHOID		1 (7%)	

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

@ 35 ANIMALS WERE INITIALLY IN THE STUDY, BUT TWO AIMALS WERE FOUND TO BE FEMALES IN A MALE GROUP.

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIESIS		1 (7%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(12)	(32)	(32)
INFLAMMATION, INTERSTITIAL	1 (8%)	1 (3%)	
INFLAMMATION, SUPPURATIVE		1 (3%)	3 (9%)
INFLAMMATION, CHRONIC SUPPURATIV			2 (6%)
FIBROSIS, DIFFUSE	1 (8%)		
NECROSIS, DIFFUSE			2 (6%)
DIGESTIVE SYSTEM			
#LIVER	(12)	(34)	(33)
THROMBOSIS, NOS		1 (3%)	
INFLAMMATION, NECROTIZING	1 (8%)		
INFLAMMATION, CHRONIC	1 (8%)		
FIBROSIS	1 (8%)		
NECROSIS, COAGULATIVE	2 (17%)		3 (9%)
CYTOPLASMIC VACUOLIZATION		1 (3%)	2 (6%)
HYPERPLASIA, NODULAR	2 (17%)	1 (3%)	1 (3%)
ANGIECTASIS		1 (3%)	
HYPERPLASIA, RETICULUM CELL		1 (3%)	
HEMATOPOIESIS		1 (3%)	
#LIVER/CENTRILOBULAR	(12)	(34)	(33)
NECROSIS, COAGULATIVE		1 (3%)	
#PANCREAS	(12)	(34)	(32)
INFLAMMATION, NECROTIZING		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(12)	(34)	(32)
CYST, NOS	1 (8%)		
INFLAMMATION, CHRONIC	1 (8%)		
FIBROSIS, FOCAL		1 (3%)	
CALCIFICATION, FOCAL		1 (3%)	
ATROPHY, NOS		1 (3%)	
ATROPHY, FOCAL	1 (8%)		
ENDOCRINE SYSTEM			
NONE			

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(12)	(34)	(33)
CYST, NOS	1 (8%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(12)	(34)	(33)
INFLAMMATION, CHRONIC		1 (3%)	
NECROSIS, FAT		2 (6%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	8	10
NO NECROPSY PERFORMED	2	1	
AUTO/NECROPSY/HISTO PERF		1	1
AUTOLYSIS/NO NECROPSY	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED L-TRYPTOPHAN IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	13	33	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	13	33	35
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(13)	(33)	(35)
INFLAMMATION, CHRONIC SUPPURATIV	1 (8%)		
#TRACHEA	(13)	(33)	(35)
INFLAMMATION, SUPPURATIVE	1 (8%)	3 (9%)	4 (11%)
#LUNG/BRONCHUS	(13)	(32)	(35)
INFLAMMATION, SUPPURATIVE	1 (8%)		
#LUNG	(13)	(32)	(35)
BRONCHOPNEUMONIA SUPPURATIVE	2 (15%)	5 (16%)	14 (40%)
PNEUMONIA INTERSTITIAL CHRONIC			1 (3%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	8 (62%)		1 (3%)
HEMOSIDEROSIS			1 (3%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (3%)	
HYPERPLASIA, PLASMA CELL		1 (3%)	3 (9%)
HYPERPLASIA, LYMPHOID		1 (3%)	1 (3%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(12)	(31)	(35)
ATROPHY, NOS			2 (6%)
#SPLEEN	(13)	(32)	(35)
INFLAMMATION, CHRONIC FOCAL			1 (3%)
ANGIECTASIS			2 (6%)
HYPERPLASIA, RETICULUM CELL		2 (6%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIESIS	1 (8%)	3 (9%)	7 (20%)
#MANDIBULAR L. NODE	(4)	(17)	(15)
HYPERPLASIA, PLASMA CELL			1 (7%)
HYPERPLASIA, RETICULUM CELL		1 (6%)	
HYPERPLASIA, LYMPHOID		1 (6%)	
#MEDIASTINAL L.NODE	(4)	(17)	(15)
THROMBOSIS, NOS		1 (6%)	
INFLAMMATION, NECROTIZING		1 (6%)	
HYPERPLASIA, PLASMA CELL			2 (13%)
HYPERPLASIA, RETICULUM CELL		1 (6%)	
#MESENTERIC L. NODE	(4)	(17)	(15)
THROMBOSIS, NOS		2 (12%)	
CONGESTION, NOS		1 (6%)	
INFLAMMATION, NECROTIZING		2 (12%)	
ATROPHY, NOS		1 (6%)	
#THYMUS	(13)	(31)	(35)
HYPERPLASIA, LYMPHOID		1 (3%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(13)	(32)	(35)
MINERALIZATION	1 (8%)		
DIGESTIVE SYSTEM			
#LIVER	(13)	(32)	(35)
THROMBOSIS, NOS			1 (3%)
INFLAMMATION, SUPPURATIVE			1 (3%)
ANGIECTASIS			2 (6%)
#PANCREAS	(13)	(33)	(35)
CYST, NOS		1 (3%)	
*RECTUM	(13)	(33)	(35)
PROLAPSE		1 (3%)	
ULCER, NOS		1 (3%)	
URINARY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#THYROID	(13)	(30)	(31)
CYSTIC FOLLICLES	1 (8%)		1 (3%)
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(13)	(32)	(34)
INFLAMMATION, SUPPURATIVE	2 (15%)	2 (6%)	2 (6%)
INFLAMMATION, CHRONIC			1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV		3 (9%)	
HYPERPLASIA, CYSTIC	11 (85%)	24 (75%)	19 (56%)
#OVARY/OVIDUCT	(13)	(32)	(34)
INFLAMMATION, CHRONIC			1 (3%)
#OVARY	(13)	(32)	(34)
CYST, NOS		5 (16%)	5 (15%)
INFLAMMATION, SUPPURATIVE			1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV		2 (6%)	2 (6%)
NERVOUS SYSTEM			
#BRAIN	(12)	(33)	(35)
HEMORRHAGE		1 (3%)	
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(13)	(33)	(35)
INFLAMMATION, SUPPURATIVE	1 (8%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (8%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(13)	(33)	(35)
HYPERPLASIA, PLASMA CELL		1 (3%)	
HYPERPLASIA, LYMPHOID		2 (6%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		2	a
NO NECROPSY PERFORMED	1	1	
AUTOLYSIS/NO NECROPSY	1	1	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS FED L-TRYPTOPHAN IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed L-Tryptophan in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma[b]	0/15 (0)	4/34 (12)	3/34 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.437	0.281
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	78
Hematopoietic System: Leukemia[b]	4/15 (27)	3/34 (9)	1/34 (3)
P Values[c,d]	P = 0.014(N)	N.S.	P = 0.026(N)
Relative Risk[f]		0.331	0.110
Lower Limit		0.058	0.002
Upper Limit		1.754	1.013
Weeks to First Observed Tumor	82	95	84

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed L-Tryptophan in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	1/11 (9)	2/30 (7)	5/30 (17)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.733	1.833
Lower Limit		0.045	0.250
Upper Limit		41.816	83.411
Weeks to First Observed Tumor	101	105	96
Thyroid: Follicular-cell Carcinoma[b]	0/14 (0)	2/33 (6)	0/32 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	--
Lower Limit		0.134	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed L-Tryptophan in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	1/14 (7)	4/33 (12)	0/32 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.697	0.000
Lower Limit		0.195	0.000
Upper Limit		80.844	8.096
Weeks to First Observed Tumor	91	100	--
Thyroid: C-cell Adenoma or Carcinoma[b]	5/14 (36)	6/33 (18)	1/32 (3)
P Values[c,d]	P = 0.004(N)	N.S.	P = 0.007(N)
Relative Risk[f]		0.509	0.088
Lower Limit		0.167	0.002
Upper Limit		1.823	0.697
Weeks to First Observed Tumor	91	100	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed L-Tryptophan in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pancreatic Islets: Islet-cell Adenoma[b]	0/15 (0)	2/33 (6)	0/34 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	--
Lower Limit		0.142	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--
Pancreatic Islets: Islet-cell Adenoma or Carcinoma[b]	0/15 (0)	2/33 (6)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.142	0.025
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed L-Tryptophan in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	13/15 (87)	31/34 (91)	21/33 (64)
P Values[c,d]	P = 0.016(N)	N.S.	N.S.
Relative Risk[f]		1.052	0.734
Lower Limit		0.878	0.595
Upper Limit		1.352	1.145
Weeks to First Observed Tumor	82	91	100

[a]Dosed groups received 25,000 or 50,000 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a dosed group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed L-Tryptophan in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	4/12 (33)	12/27 (44)	9/32 (28)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.333	0.844
Lower Limit		0.543	0.313
Upper Limit		4.670	3.218
Weeks to First Observed Tumor	103	75	92
Pituitary: Chromophobe Carcinoma[b]	0/12 (0)	1/27 (4)	2/32 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.025	0.120
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	86	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed L-Tryptophan in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b]	4/12 (33)	13/27 (48)	11/32 (34)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.444	1.031
Lower Limit		0.601	0.408
Upper Limit		4.964	3.779
Weeks to First Observed Tumor	103	75	92
Thyroid: Follicular-cell Carcinoma[b]	0/14 (0)	1/32 (3)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	--
Lower Limit		0.025	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed L-Tryptophan in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	1/14 (7)	2/32 (6)	1/33 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.875	0.424
Lower Limit		0.051	0.006
Upper Limit		49.995	32.328
Weeks to First Observed Tumor	105	105	104
Mammary Gland: Adenoma, NOS (not otherwise specified)[b]	0/14 (0)	0/35 (0)	2/34 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		--	Infinite
Lower Limit		--	0.130
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed L-Tryptophan in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	4/14 (29)	7/35 (20)	2/34 (6)
P Values[c,d]	P = 0.027(N)	N.S.	N.S.
Relative Risk[f]		0.700	0.206
Lower Limit		0.224	0.022
Upper Limit		2.873	1.289
Weeks to First Observed Tumor	96	91	92
Mammary Gland: Adenoma or Fibroadenoma[b]	4/14 (29)	7/35 (20)	4/34 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.700	0.412
Lower Limit		0.224	0.094
Upper Limit		2.886	1.957
Weeks to First Observed Tumor	--	--	92

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed L-Tryptophan in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	3/13 (23)	3/35 (9)	3/33 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.371	0.394
Lower Limit		0.060	0.064
Upper Limit		2.516	2.664
Weeks to First Observed Tumor	105	105	104

[a]Dosed groups received 25,000 or 50,000 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a dosed group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE FED L-TRYPTOPHAN IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed L-Tryptophan in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	0/12 (0)	3/33 (9)	2/32 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.237	0.120
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	74	102
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/12 (0)	4/33 (12)	2/32 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.367	0.120
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	74	102

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed L-Tryptophan in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	0/12 (0)	9/34 (26)	2/33 (6)
P Values[c,d]	N.S.	P = 0.048	N.S.
Departure from Linear Trend[e]	P = 0.005		
Relative Risk[f]		Infinite	Infinite
Lower Limit		1.016	0.117
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	62	67
Trigeminal Ganglion: Malignant Neurilenoma[b]	0/12 (0)	2/34 (6)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	--
Lower Limit		0.113	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	67	--

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed L-Tryptophan in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma[b]	1/12 (8)	4/34 (12)	5/33 (15)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.412	1.818
Lower Limit		0.165	0.143
Upper Limit		67.331	83.089
Weeks to First Observed Tumor	104	94	102
Liver: Hepatocellular Carcinoma[b]	0/12 (0)	1/34 (3)	2/33 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.020	0.117
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	100	104

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed L-Tryptophan in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	1/12 (8)	5/34 (15)	7/33 (21)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.765	2.545
Lower Limit		0.236	0.396
Upper Limit		80.745	110.503
Weeks to First Observed Tumor	104	94	102

[a]Dosed groups received 25,000 or 50,000 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a dosed group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed L-Tryptophan in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	2/13 (15)	6/33 (18)	1/35 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.182	0.186
Lower Limit		0.256	0.003
Upper Limit		11.065	3.348
Weeks to First Observed Tumor	92	67	61

[a]Dosed groups received 25,000 or 50,000 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a dosed group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

Review of the Bioassay of L-Tryptophan for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

March 7, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of L-Tryptophan for carcinogenicity.

The primary reviewer agreed with the conclusion in the report that, under the conditions of test, L-Tryptophan was not carcinogenic in rats or mice. After a brief description of the experimental design and conditions of test, he said that the study was adequate to support this conclusion.

As the secondary reviewer, Dr. Kuschner noted that others have reported tumors in mice administered L-Tryptophan. He questioned the use of rodents as an appropriate species for studying the carcinogenicity of L-Tryptophan and suggested that the dog would be a more relevant experimental model.

A motion was made that the report on the bioassay of L-Tryptophan be accepted as written. The motion was seconded and approved unanimously.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology

Arnold Brown, Mayo Clinic
E. Cuyler Hammond, American Cancer Society
Joseph Highland, Environmental Defense Fund
Henry Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Michael Shimkin, University of California at San Diego

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

DHEW Publication No. (NIH) 78-1321